Data Evaluation Report on the Acute Toxicity of RPA 410193 (S-Enantiomer of RPA 405862) to Freshwater Invertebrates - *Daphnia magna*

PMRA Submission Number{.....} EPA MRID Number 45385726

Data Requirement: PMRA DATA CODE:{.....}
EPA DP Barcode: D275213
OECD Data Point:
EPA MRID: 45385726
EPA Guideline: 72-2

DEGRADATE OF FENAMIDONE 046679

Test material: RPA 410193 **Purity:** 99.9%
Common name: S-Enantiomer of RPA 405862
Chemical name: (S)-5-methyl-5-phenyl-3-phenylaminoimidazolidine-2,4-dione
CAS name: (S)-5-methyl-5-phenyl-3-phenylaminoimidazolidine-2,4-dione
CAS No.: Not reported
Synonyms: RPA 410193

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Reference/Submission No. {.....}

Company Code {.....} [For PMRA]
Active Code {.....} [For PMRA]
EPA PC Code 046679

Date Evaluation Completed: {dd-mmm-yyyy}

CITATION: Odin-Feurtet, M. 1999. RPA 410193 (S-Enantiomer of RPA 405862) Acute toxicity (48 hours) to daphnids *(Daphnia magna)* under semi-static conditions. Unpublished study performed by Rhône-Poulenc Agro, Centre de Recherche, 355, rue Dostoïevski, BP 153, F-06903 Sophia Antipolis Cedex and sponsored by Rhône-Poulenc Agro, 14-20, rue Pierre Baizet, BP 9163, F-69263 Lyon Cedex 09. Study number: SA 99016. Study initiated on January 27, 1999 and completed on April 01, 1999.

EXECUTIVE SUMMARY:

The 48-hr-acute toxicity of RPA 410193 to *Daphnia magna* was studied under static renewal conditions. Dilution water control served as a comparison to five mean measured concentrations of RPA 410193: 0 (control), 0 (solvent), 1.4, 3.1, 5.5, 12.4 and 21.1 mg/L. The two highest concentrations had precipitates, therefore, the available chemical could not be determined. The 48-hour EC_{50} was >5.5 mg/L, the highest level without a precipitate. As a result, RPA 410193 is classified as moderately toxic to *Daphnia magna* on an acute toxicity basis. The 48-hr- NOAEC based on sublethal effects was 5.5 mg a.i./L.

This study is scientifically sound, but does not satisfy the guideline requirements for an acute toxicity study with freshwater invertebrates. An EC50 was not determined. This study is classified as supplemental for this degradate of fenamidone.

Results Synopsis

Test Organism Age (*e.g.*, 1st instar): ≤24 hrs. old
Test Type: Static Renewal

24- hour EC_{50} : >5.5 mg/L 95% C.I.: N/A
48- hour EC_{50} : >5.5 mg/L 95% C.I.: N/A
NOAEC (48 hours): 5.5 mg/L

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: OECD guideline N° 202 I (1984), E.E.C. directive 92/69- method C2 (1992) and E.P.A./FIFRA guideline 72-2 (1985).

Deviations included:

1. Water hardness (164-166 mg/L $CaCO_3$) was significantly higher than recommended (40-48 mg/L as $CaCO_3$) and th pH (7.83-8.02) was higher than recommended (7.2-7.6).

2. Precipitate was observed in test solutions from the two highest concentrations (12.4 and 21.1 mg/L) and there was no indication that test solutions were centrifuged or filtered.

3. The study author failed to report the OECD test chemical physical characteristics (i.e., water solubility, vapor pressure, and specific activity).

4. The study author failed to indicate the level of analytical detection.

5. Loading rate not described.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

A. MATERIALS:

1. Test Material RPA 410193 ((S)-5-methyl-5-phenyl-3-phenylaminoimidazolidine-2,4-dione)

Description: White powder
Lot No. : YG2965
Purity: 99.9%

Stability of Compound Under Test Conditions: Measured RPA 410193 concentrations of fresh solutions (0 and 24 hours) were 75-100% of nominal concentrations and measured concentrations of expired solutions (24 and 48 hours) were 93-103% of measured concentrations in fresh solutions, showing that the test material was stable under test conditions. OECD requirements were not reported.

Water solubility: Not reported
Vapor pressure: Not reported
Specific activity: Not reported
Molecular weight: 281.317 g/mol

(OECD requires water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound)

Storage conditions of test chemicals: The test material was stored in the dark in an airtight container, at room temperature (approximately 20°C).

2. Test organism:

Species: *Daphnia magna* *EPA preferred species is Daphnia magna*
Age at test initiation: <24 hrs. old
Source: Clone 5 originating from INERIS Laboratory (BP1-91710 Vert-le-petit, France).

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: A range finding study was performed under static renewal conditions with a dilution water control, a solvent control and four nominal test concentrations of RPA 410193 (3.13, 6.25, 12.5 and 25.0 mg/L). No immobilization was observed in the dilution water control, solvent control and treatment groups at test termination.

b) Definitive Study

Table 1 . Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period:	Daphnids used in this toxicity test were obtained from laboratory cultures and were less than 24 hours old.	Dilution water was same as culture water
Conditions: (same as test or not)	Same as test	
Feeding:	*Daphnia* cultures were fed a combination of flake fish food (Tetramin), nutrient broth, yeast suspension, seaweed extract (Marinure 30) and unicellular green algae (*Chlorella vulgaris*) three times weekly. During the course of the study *Daphnia* were not fed.	*EPA requires 7 day minimum acclimation period* *No feeding during study*
Health: (any mortality observed)	Prior to the test period *Daphnia* were healthy. Before 2 days of the study period, < 3% of mortality was observed in the brood culture	
Duration of the test	48 hours	*EPA requires 48 hours*

Parameter	Details	Remarks *Criteria*
Test condition static/flow through Type of dilution system- for flow through method. Renewal rate for static renewal	Static renewal test N/A 24 hours	
Aeration, if any	Prior to the study period, the culture medium were continuously aerated (air bubbling). The test solutions were not aerated.	
<u>Test vessel</u> Material: *(glass/stainless steel)* Size: Fill volume:	 Glass 250 mL 200 mL	 *EPA requires: size 250 ml or 3.9 L fill 200 ml*
Source of dilution water	Reconstituted water 80% DSW + 20% LC-oligo; see Appendix 2, pp. 33-35)	*EPA requires soft reconstituted water or water from a natural source, not dechlorinated tap water.*
<u>Water parameters</u>: Hardness pH Dissolved oxygen Temperature Total Organic Carbon Particulate matter Metals Pesticides Chlorine	 164-166 mg/L $CaCO_3$ 7.83-8.02 ≥7.9 mg/L 20.1°C-20.9°C Not reported <1 mg/L pp. 33-34 p. 35 Not reported	Water hardness was higher than required by EPA. The pH was higher than recommended. *EPA requires:* *hardness: 40 - 48 mg/L as $CaCO_3$* *pH: 7.2 - 7.6* *-Temperature: 20°C (measured continuously or if water baths are used, every 6 hr, may not vary > 1°C* *Dissolved oxygen:* *Static: ≥ 60% during 1st 48 hr and ≥ 40% during 2nd 48 hr* *Flow-through: ≥60%*
Number of replicates Solvent control: Negative control: Treatments:	 1 1 2	

Parameter	Details	Remarks *Criteria*
Number of organisms per replicate Solvent control: Negative control: Treatments:	 1 1 10 per replicate; 2 replicates per treatment level	Five treatment levels plus water control with 20 *Daphnia* per treatment. *EPA requires 5 treatment levels plus control with a minimum of 20 daphnid per treatment. Biomass loading rate for static ≤ 0.8 g.L at ≤ 17oC, ≤ 0.5 g/L at > 17oC; flow-through: ≤ 1 g/L/day.*
Treatment concentrations nominal: measured:	 Water control, solvent control, 1.6, 3.1, 6.3, 12.5 and 25.0 mg a.i./L RPA 410193 Water control, solvent control, 1.4, 3.1, 5.5, 12.4 and 21.1 mg a.i./L RPA 410193	Mean measured concentrations are the average of samples analyzed in fresh and expired solutions over the 48 hour study period. *EPA requires a geometric series with each concentration being at least 60% of the next higher one.*
Solvent (type, percentage, if used)	Dimethylformamide (0.1 mL/L)	 *EPA requires solvents not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-though tests.*
Lighting	16 hours light and 8 hours dark	 *EPA requires 16 hours light, 8 hours dark.*
Stability of chemical in the test system	Measured RPA 412636 concentrations of fresh solutions (0 and 24 hours) were 75-100% of nominal concentrations and measured concentrations of expired solutions (24 and 48 hours) were 93-103% of measured concentrations in fresh solutions	

Parameter	Details	Remarks *Criteria*
Recovery of chemical	75-100% of nominal concentrations; 93-103% of freshly measured solutions	
Level of Quantitation	0.2 mg/L	
Level of Detection	Not reported	
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	N/A	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks *Criteria*
Parameters measured including the sublethal effects	Sublethal effects (immobilization)	
Observation intervals	Daily	
Were raw data included?	Yes	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION

A. SUB-LETHAL TOXICITY ENDPOINTS:

No immobilization was observed in any treatment group over the 48 hour study period.

Table 3: Effect of RPA 410193 on the immobilization of *Daphnia magna.*

Measured (and Nominal) Treatment Concentrations (mg/L)	Observation period			
	Day 24		Day 48	
	endpoint	% affected	endpoint	% affected
Dilution water control	Immobilization	0	Immobilization	0
Solvent control	Immobilization	0	Immobilization	0
Positive control, if used	N/A	N/A	N/A	N/A
1.4 (1.6)	Immobilization	0	Immobilization	0
3.1 (3.1)	Immobilization	0	Immobilization	0
5.5 (6.3)	Immobilization	0	Immobilization	0
12.4 (12.5)	Immobilization	0	Immobilization	0
21.1 (25.0)	Immobilization	0	Immobilization	0
NOAEC mg a.i./L	5.5 mg/L		5.5 mg/L	
LOAEC	5.5 mg/L		5.5 mg/L	
EC_{50} mg a.i./L	5.5 mg/L		5.5 mg/L	

C. REPORTED STATISTICS:

Statistical analyses were not required, as no immobility was observed.

LC_{50} : N/A 95% C.I.: N/A
24- hour EC_{50} : >21.1 mg/L 95% C.I.: N/A
48- hour EC_{50} : >21.1 mg/L 95% C.I.: N/A
NOAEC (48 hours): 21.1 mg/L

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical analyses were not required, as no immobility was observed.

LC_{50} : N/A 95% C.I.: N/A
24- hour EC_{50} : >5.5 mg/L 95% C.I.: N/A
48- hour EC_{50} : >5.5 mg/L 95% C.I.: N/A
NOAEC (48 hours): 5.5 mg/L

E. STUDY DEFICIENCIES:

The water hardness in this study was substantially higher (164-166 mg/L $CaCO_3$) than that recommended by US EPA (40-48 mg/L $CaCO_3$) and the pH of the test solution also varied outside the range recommended by US EPA. Furthermore, precipitate was observed in solutions of the two highest test concentrations and there was no report of centrifugation or filtration prior to analysis. Because these factors may influence the bioavailability of toxins to daphnids, these deviations impacted the acceptability of this study.

F. REVIEWER'S COMMENTS:

The study author reported that precipitate was observed in test solutions from the two highest concentrations (12.4 and 21.1 mg a.i./L), indicating that they were above the visual limit of aqueous solubility. They also reported that the precipitate and particles disappeared over time. US EPA recommends centrifugation or filtration of all test solutions with the appearance of precipitate to enhance solubility and there was no indication that this was done. Therefore, the two highest concentrations were not considered in the analysis. This study is Supplemental.

G. CONCLUSIONS:

This study is scientifically sound and but does not fulfil EPA guidelines for toxicity testing with freshwater invertebrates (§72-2). This study is classified as Supplemental, because only three concentrations that could be analyzed and no EC50 could be determined. The 48-hour EC_{50} was >5.5 mg/L, which classifies RPA 410193 as no more than moderately toxic to daphnids on an acute toxicity basis. The NOAEC was 5.5 mg a.i./L.

24- hour EC_{50} : >5.5 mg/L 95% C.I.: N/A
48- hour EC_{50} : >5.5 mg/L 95% C.I.: N/A
NOAEC (48 hours): 5.5 mg/L

III. REFERENCES:

1) ASTM (1994) - Standard guide for conducting acute toxicity tests with fishes, macroinvertebrates and amphibians. E729-88a, American Society for Testing and Materials, 1916 Race Street, Philadelphia, PA, 19103 - 1187, U.S.A.

2) E.E.C. (1992) - Annex to Commission Directive 92/69/E.E.C. of 31/07/92 - Part C,

Methods of determination of Ecotoxicity - Method C2: Acute toxicity to daphnids, Official Journal of European Communities, Publication n° L 383 A, pp 172-178.

3) US E.P.A. (1985): Hazard Evaluation Division - Standard Evaluation Procedure - Acute toxicity test for freshwater invertebrates (EPA540/9-85-005), FIFRA guideline n°72-2.

4) O.E.C.D. (1984) - Guidelines for testing of Chemicals - Section 2: Effects on biotic systems: 202 - *Daphnia sp.*, Acute immobilisation test and reproduction test - Part I - The 24-hour EC_{50} acute immobilization test.

5) RPA 410193: determination by High Performance Liquid Chromatography. Analysis in freshwater for ecotoxicology: ANL/197-99E, J.P. Oullier, J. P. Tassel, Rhône-Poulenc Agro, Sophia Antipolis Research Center, 1999.